Bohn, Brent

From: Kloc, Kenneth@OEHHA <Kenneth.Kloc@oehha.ca.gov>
Sent: Friday, July 31, 2015 5:45 PM
To: Sasso, Alan
Subject: RE: n-Butanol report reference

Hi Alan,
Thanks very much for sending the available materials. They will be helpful in our review work.
-Ken

From: Sasso, Alan [mailto:Sasso.Alan@epa.gov]
Sent: Friday, July 31, 2015 6:59 AM
To: Kloc, Kenneth@OEHHA
Subject: RE: n-Butanol report reference

Ken,

Attached are the only translated papers I could find, along with a memo summarizing some of the papers (which may have been reviewed by other agencies, but not translated by us). I've attached all the original Russian copies as well.

I couldn't find a translated copy of Baikov, although the paper is summarized in the attached memo. In our document, that citation is associated with a detailed table. Maybe the data are published in older reviews by other agencies ("WHO (1987) and MOE (2007)"). Data may have also been extracted from the graph in the Russian version.

I found a translated paper by Rumyantsev, but it's from 1979. It may be related to the 1976 paper (it could contain data or results from 1976, and the previous manager may have decided to only translate the later paper to save resources).

I also found a different paper that we translated (Kolesnikov 1975). I'm not sure if it's related, but I figured I would send it anyway.

I hope this helps. We will keep an eye out for correct translations and will let you know if we find anything.

-Alan

From: Kloc, Kenneth@OEHHA [mailto:Kenneth.Kloc@oehha.ca.gov]
Sent: Thursday, July 30, 2015 4:26 PM
To: Sasso, Alan
Subject: RE: n-Butanol report reference

Much appreciated!
Ken

From: Sasso, Alan [mailto:Sasso.Alan@epa.gov]
Sent: Thursday, July 30, 2015 1:13 PM
To: Kloc, Kenneth@OEHHA
Subject: RE: n-Butanol report reference

Hi Dr. Kloc,

I'm currently trying to find the translations, and will let you know as soon as possible.

The previous chemical manager of this chemical (Ambuja Bale) has left the agency, so I will need to look through her archived files.

-Alan

Alan F. Sasso, Ph.D.
Office of Research and Development
National Center for Environmental Assessment
U.S. Environmental Protection Agency
(703)-347-0179

From: Kloc, Kenneth@OEHHA [mailto:Kenneth.Kloc@oehha.ca.gov]
Sent: Thursday, July 30, 2015 3:33 PM
To: Sasso, Alan
Subject: n-Butanol report reference

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Or please feel free to let me know if you cannot fulfill this request.

Best Regards,
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Or please feel free to let me know if you cannot fulfill this request.

Best Regards,
Ken Kloc, Ph.D.

Title: Sensitivity analysis of internal dose-metrics for hexavalent chromium toxicity using physiologically-based pharmacokinetic modeling

Alan F. Sasso, Paul M. Schlosser

U.S. Environmental Protection Agency, National Center for Environmental Assessment

Hexavalent chromium (Cr(VI)) is an environmental and occupational contaminant, and is present in both soil and drinking water in the United States. In 2-year drinking water bioassays, the National Toxicology Program observed effects including carcinogenicity of the gastrointestinal (GI) tract in mice and oral cavity in rats. Physiologically-based pharmacokinetic (PBPK) models have been developed to estimate interspecies differences in toxicity and assess human health risk from oral exposure to Cr(VI). However, there are significant uncertainties and inter-individual variabilities to consider when modeling chromium in the gastrointestinal tract. Hexavalent chromium is rapidly reduced to trivalent chromium (Cr(III)) in the GI lumen, and only total chromium can be analytically measured in vivo. The reduction and absorption of hexavalent chromium will vary with intestinal pH, dietary intake, and gastric contents and physiology. These factors vary over time and between individuals. In addition, multiple PBPK models have been developed for hexavalent chromium, and a variety of different internal dose-metrics may be applied to link external exposure and toxic effects. Toxicity may be correlated to 1) concentration of Cr(VI) in the GI tract lumen; 2) absorption of Cr(VI) into specific GI tract tissue sites; 3) total absorption of Cr(VI) in the full GI tract. This work quantifies the impact of different modeling assumptions on the interpretation of toxicological data in rodents, and extrapolation to humans. It was found that the choice of dose-metric has a significant impact on the evaluation of potential human health effects from oral exposure to hexavalent chromium.

From: Sasso, Alan
Sent: Monday, February 10, 2014 10:09 AM
To: Elaine.Khan@oehha.ca.gov; Gibbons, Catherine
Subject: RE: Cr6 PBPK Model
Attachments: Kanojia-Junaid-compare.pdf; Junaid-etal_BulletEnvContTox1996_embryo-fetotoxicity-mice.pdf; Kanojia-etal_ToxLett1996_chromium-teratogenicity-rat.pdf

Hi Elaine,

I know this is somewhat off-topic, but it's a notable scientific issue and the opinions of your group would be very helpful.

We, with the help of our contractor ICF, found that two datasets published by the Junaid/Kanojia/Murthy/Saxena investigators are essentially identical, despite being published as separate studies for separate species (one in rats, one in mice). We have contacted the journals and they are investigating it.

Kanojia, RK; Junaid, M; Murthy, RC. (1996). Chromium induced teratogenicity in female rat. Toxicology letters 89: 207-213.

Junaid, M; Murthy, RC; Saxena, DK. (1996). Embryo- and fetotoxicity of chromium in pregestationally exposed mice. Bulletin of environmental contamination and toxicology 57: 327-334.

The study in Toxicology Letters cites the study in Bulletin of Environmental Contamination and Toxicology as a separate study. See the attached "Kanojia-Junaid-compare.pdf" document for a quick comparison.

Whether or not this is an academic integrity issue, or simply a case of sloppy recordkeeping, we are unable to determine which of the two tables are "correct", and thus don't know which species the endpoints really correspond to.

These investigators have numerous other follow-up papers, and these are frequently cited as evidence for this host of endpoints by ATSDR and your own assessments.

If we hear back from the journals about their decision of whether to amend or retract the studies, we will let you know.

-Alan

From: Khan, Elaine@OEHHA [mailto:Elaine.Khan@oehha.ca.gov]
Sent: Wednesday, February 05, 2014 6:03 PM
To: Gibbons, Catherine; Sasso, Alan
Subject: RE: Cr6 PBPK Model

Thanks, Catherine! No rush on the meeting – Patty (our PBPK guru-in-training) will be busy wrapping up a project over the next 3 weeks or so. If your schedule looks flexible in March, we can shoot for some time then. Just let me know. Thanks!

Elaine

From: Gibbons, Catherine [mailto:Gibbons.Catherine@epa.gov]
Sent: Wednesday, February 05, 2014 10:22 AM

To: Khan, Elaine@OEHHA; Sasso, Alan
Subject: RE: Cr6 PBPK Model

Hi Elaine!

I was just checking my phone messages and heard your message from a few weeks ago—I've been out of town a lot recently—but I never received a signal that I had a message, I apologize for the delay! But I'm glad you wrote.

Alan and I would be happy to set up a time for a call. I'll discuss possible times/days with Alan and get back to you as quickly as possible.

Thanks so much!

Catherine

From: Khan, Elaine@OEHHA [mailto:Elaine.Khan@oehha.ca.gov]
Sent: Tuesday, February 04, 2014 3:10 PM
To: Sasso, Alan; Gibbons, Catherine
Subject: RE: Cr6 PBPK Model

Hi, Alan.

Yes, Mark was referring to your presentation at SRA in Baltimore. Thank you for sending your talk and abstract to us. I will only share this internally with my staff and executive office as needed (it will not be cited). I look forward to having a discussion with you and Catherine soon.

Elaine

From: Sasso, Alan [mailto:Sasso.Alan@epa.gov]
Sent: Tuesday, February 04, 2014 11:08 AM
To: Khan, Elaine@OEHHA; Gibbons, Catherine
Subject: RE: Cr6 PBPK Model

HI Elaine,

A conference call would be great. When Catherine comes back to the office later this week, we'll be able to schedule one soon.

Mark was probably referring to the talk I gave at the Society for Risk Analysis conference. I have attached that talk, along with the abstract for a poster I plan on presenting at the Society of Toxicology meeting in March.

The material has not yet been peer reviewed, so please do not distribute or cite the materials.

Thanks and take care,

-Alan

Alan F. Sasso, Ph.D.
Office of Research and Development
National Center for Environmental Assessment
U.S. Environmental Protection Agency

(703)-347-0179

From: Khan, Elaine@OEHHA [mailto:Elaine.Khan@oehha.ca.gov]
Sent: Tuesday, February 04, 2014 1:14 PM
To: Gibbons, Catherine; Sasso, Alan
Subject: Cr6 PBPK Model

Hi, Catherine and Alan.

I hope your year has gotten off to a good start so far! I've been keeping in touch with Mark Harris (ToxStrategies) regarding their Cr6 studies and he informed me that they provided you with additional PBPK information, which you used to build your own model. I was wondering if we could set up a conference call sometime soon to touch base on the Cr6 assessment. We're very interested in seeing how your PBPK model differs from theirs. Please let me know when it would be convenient for us to have a meeting. Thanks!

Elaine

Elaine M. Khan, Ph.D., Chief
Water Toxicology Section
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency
MS-12B
P.O. Box 4010
1001 I Street
Sacramento, CA 95812
Tel: (916) 324-1277
Fax: (916) 327-7320
Email: elaine.khan@oehha.ca.gov

Table 2
Incidences of gross and skeletal abnormalities in the pups of chromium-treated rats during the pregestational period

Parameter	Group I (control)	Group II (250 ppm)	Group III (500 ppm)	Group IV (750 ppm)
Gross abnormalities				
Number of pups/litter observed	72/10	70/10	51/10	19/10
Drooping wrist	0	0	0	6/4 (32)
Sub-dermal hemorrhagic patches	0	0	8/6 (16)	8/4 (42)[a]
Kinky tail	0	0	0	8/6 (42)[a]
Short tail	0	0	4/4 (9)	10/4 (53)[a]
Skeletal abnormalities				
Number of pups/litter observed	48/10	45/10	34/10	19/10
Reduced parietal ossification	0	0	0	12/10 (63)[a]
Reduced inter-parietal ossification	0	0	0	10/10 (53)[a]
Reduced caudal ossification	6/4 (12)	8/5(18)	18/8 (53)[a]	18/10 (95)[a]

Gross and skeletal abnormalities are represented as number of abnormal pups/litter observed; percentage in parentheses calculated by the total number of pups observed.
Statistical significance evaluated by Fisher's Exact test; comparison between two groups. [a]vs. control. Significance level: $p < 0.05$.

Above: Kanojia, RK; Junaid, M; Murthy, RC. (1996). Chromium induced teratogenicity in female rat. Toxicol Lett 89: 207-213.

Below: Junaid, M; Murthy, RC; Saxena, DK. (1996). Embryo and fetotoxicity of chromium in pregestationally exposed mice. Bull Environ Contam Toxicol 57: 327-334.

Table 2. Incidences of gross and skeletal abnormalities in the pups of dams treated with chromium during the pregestational period.

Parameters	Group I (Control)	Group II (250 ppm)	Group III (500 ppm)
Gross abnormalities			
Number of pups/litters observed	72/10	51/10	19/10
Drooping rist	0/10	0/10	6/4 (32)
Sub-dermal hemorrhagic patches	0	8/6 (16)	8/4 (42) a*
Kinky tail	0	0	8/6 (42) a*
Short tail	0	4/4 (9)	10/4 (53) a*
Skeletal abnormalities			
Number of pups/litter observed	48/10	34/10	19/10
Reduced parietal ossification	0	0	12/10 (63) a*
Reduced inter-parietal ossification	0	0	10/10 (53) a*
Reduced caudal ossification	6/4 (12)	18/8 (53) a*	18/10 (95) a*

Gross and skeletal abnormalites are represented as number of abnormal pups/litters observed.

The statistical significance was evaluated by Fisher's Exact test (Drunning and Kintz 1977).

Percentage in parentheses calculated by the total number of pups observed.

* Significance $p < 0.05$. Comparison between two groups: a-vs control.

Table 3
Chromium concentrations in different tissues of rats treated during the pregestational period

Tissue	Group I (control)	Group II (250 ppm)	Group III (500 ppm)	Group IV (750 ppm)
Blood (μg/ml)	0.034 ± 0.007	0.049 ± 0.006[a]	0.059 ± 0.008[a]	0.192 ± 0.007[abc]
Placenta (μg/g: fw)	0.093 ± 0.001	0.151 ± 0.008[a]	0.168 ± 0.002[ab]	0.232 ± 0.019[abc]
Fetus (μg/g: fw)	0.042 ± 0.008	0.069 ± 0.007	0.163 ± 0.013[ab]	0.241 ± 0.011[abc]

Values represent mean ± S.E. of five rats in each group; fw, fresh weight.
Significance of the difference among various groups was evaluated by applying one-way ANOVA. Significance level: $p < 0.05$; comparison between two groups: [a]vs. control; [b]vs. 250 ppm; [c]vs. 500 ppm.

Above: Kanojia, RK; Junaid, M; Murthy, RC. (1996). Chromium induced teratogenicity in female rat. Toxicol Lett 89: 207-213.

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Table 3. Chromium concentrations in different tissues of mice treated during the pregestational period

Tissue	GroupI (Control)	Group II (250 ppm)	Group III (500 ppm)	Group IV (750 ppm)
Blood (μg/mL)	0.03 + 0.007	0.05 ± 0.006 a*	0.06 ± 0.008 a*	0.13 ± 0.007 abc*
Placenta (μg/g:f.w.)	0.09 ± 0.001	0.14 ± 0.008 a*	0.17 ± 0.002 ab*	No implantation
Fetus (μg/g:f.w.)	0.04 ± 0.008	0.07 + 0.007	0.16 ± 0.013 ab*	No implantation

Values represent mean ± S.E of 5 mice in each group.

The significance of the difference among various groups was evaluated by applying one-way ANOVA followed by Student's 't' test (Brunning and Kintz 1977). * Significance p < 0.05.

Comparison between two groups: a -vs control; b -vs 250 ppm; c -VS 500 ppm. f.w. q fresh weight.

ELSEVIER

Toxicology Letters 89 (1996) 207–213

Toxicology Letters

Chromium induced teratogenicity in female rat

Raj Kamal Kanojia, Mohammad Junaid, Ramesh Chandra Murthy*

Metal Analysis Laboratory, Industrial Toxicology Research Centre, Post Box 80, M.G. Marg, Lucknow-226001, India

Received 17 May 1996; revised 12 August 1996; accepted 13 August 1996

Abstract

Exposure to chromium (VI) (250, 500 and 750 ppm as potassium dichromate) via drinking water pregestationally in rats revealed embryo- and fetotoxic effects in the form of a significant reduction in the number of implantations and number of fetuses. An increase in the number of resorptions, pre-implantation and post-implantation loss in chromium (VI)-treated mothers was also observed. No significant visceral abnormality was found. A significant increase in sub-dermal hemorrhagic patches on thoracic and abdominal areas was found. Skeletal abnormality in the form of reduced ossification in parietal, interparietal and caudal bones was found in the fetuses of chromium (VI)-treated mothers. Chromium levels in blood, placenta and fetuses were found to be significantly increased in the 500 ppm and 750 ppm dosed groups. The duration of estrus cycle was significantly altered after chromium (VI) exposure. This study suggests that chromium exposure in rat causes a lower degree of toxicity than in mice as observed in our earlier studies.

Keywords: Hexavalent chromium; Drinking water; Pregestational period; Teratogenicity; Rats

1. Introduction

Chromium, an essential element for biological systems is also used in metallurgical processes, chrome plating, pigment production, tanning, textile, ceramic, glass and photographic industries. High concentrations of chromium (40–50 000 ppm) have been reported in the effluents from these industries [1]. Besides exposure to industrial workers, the general population is also exposed to this metal as it contaminates surface and ground water, agricultural land and aquatic life [2,3]. High levels of chromium are reported to impair gestational development as evidenced by epidemiological studies in female workers exposed to this metal in the work environment [4]. Exposure to chromium (VI) resulted in complications during pregnancy and childbirth in the form of toxicosis and puerperal hemorrhages in women employees at a dichromate manufacturing factory [5]. Tipton [6] reported the transfer of chromium from the

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PII S0378-4274(96)03812-X

mother to the bones of the developing fetus in humans. Pribluda [7] reported that the chromium content of bones of pregnant rats decreases with advancing gestation. Such released chromium may reach the circulatory system and enter feto-placental tissues through the placental barrier.

Our earlier study [8] revealed developmental changes in mice after oral exposure to chromium (VI) pregestationally. However no study to date has been carried out to determine the effect of chromium in rats exposed pregestationally. A significant difference in the feto-placental barrier of the two species (mice and rats) was observed, with mouse fetoplacental unit allowing a greater inflow of chromium (VI) from maternal blood to the fetuses whereas in rats the feto-placental barrier, to a greater extent, restricted the inflow of orally administered chromium (VI) [9]. Therefore, the present study was carried out to determine the effect of chromium (VI) on embryo-fetal development in rats exposed orally during the pregestational period of development. In addition, we wanted to establish the relative species susceptibility and also determine the distribution of chromium (VI) in the maternal and feto-placental unit.

2. Materials and methods

Adult Swiss albino female rats (120 days old; body weight 175 ± 25 g) of proven fertility from the Industrial Toxicology Research Centre bred colony were taken, synchronised for cyclicity and were divided into four equal groups. Group I was given tap water (Chromium level < 0.001 ppm) and served as controls. The remaining groups (group II, III and IV) were given 250, 500 or 750 ppm chromium (VI) [as potassium dichromate; AR, 99.9% pure, Ranbaxy Laboratories Ltd., India], respectively, for 20 days [one folliculogenesis cycle [8]]. The dose was selected on the basis of our earlier studies [8,10,11]. After the completion of the treatment, they were kept for mating (1:1) with normal healthy adult males overnight. The presence of sperm in the vaginal smear was designated as day '0' of gestation. The animals were kept in plastic cages individually under standard animal care conditions.

They were provided with pellet feed (Cr level 1.45 μg/g; Lipton India Ltd.) and water ad libitum. The body weight and water intake were recorded daily. Mating and fertility indices were calculated from the formulae:

$$\text{Mating index (\%)} = \frac{\text{No. of females kept for mating} - \text{number of mated females}}{\text{No. of females kept for mating}}$$

$$\text{Fertility index (\%)} = \frac{\text{No. of females mated} - \text{No. of pregnant females}}{\text{No. of females mated}}$$

Cesarian sections were performed on day 19 of gestation in 10 animals from each group. Blood was withdrawn from the heart and kept at -20°C for chromium estimation. Ovaries were removed, the number of corpora lutea counted, and number of fetuses/litter, number of live/dead fetuses, crown-rump length, number of resorptions, weight of fetuses with their respective placentae were recorded. Pre- and post-implantation loss was calculated as described by Palmer et al. [12]. One fetus/litter with its placenta was kept at -20°C for chromium estimation. One-third of the remaining fetuses were fixed in Bouin's fluid for examining the visceral abnormalities [13]. The remainder of the fetuses from each group were first examined for gross external abnormalities and then were fixed in 95% ethanol, eviscerated and stained by the Alizarin red S method [14] for examining skeletal deformities [15].

2.1. Chromium estimation

Maternal blood was measured, placenta and fetuses were washed with saline, blotted dry and weighed, then digested in a $HNO_3/HClO_4$ (6:1) mixture until a white residue remained. This residue was dissolved in an appropriate amount of 0.1 N HNO_3 and chromium was estimated on a DC Plasma Emission Spectrophotometer (Beckman Spectrospan V). Blank and chromium-spiked samples were run and analyzed simultaneously [16,17].

Table 1
Chromium-induced embryo- and feto-toxicity in rats treated during pregestational period

Parameter	Group I (control)	Group II (250 ppm)	Group III (500 ppm)	Group IV (750 ppm)
Mating Index (%)	100	80	70	40
Fertility Index (%)	96	75	57	31
Weight gain in mothers (g)	70.50 ± 5.19	65.02 ± 3.17[a]	60.92 ± 2.13[ab]	55.5 ± 3.01[abc]
Number of corpora lutea	10.02 ± 0.91	9.81 ± 0.95	7.13 ± 0.61[ab]	4.43 ± 0.50[abc]
Number of implantations	9.51 ± 0.96	9.61 ± 0.83	5.91 ± 0.39[ab]	2.27 ± 0.36[abc]
Number of live fetuses	9.11 ± 0.87	8.29 ± 0.93[a]	4.12 ± 0.51[ab]	1.21 ± 0.13[abc]
Number of resorptions	0.40 ± 0.24	1.09 ± 0.34[a]	1.72 ± 0.23[a]	1.03 ± 0.29[a]
Pre-implantation loss	5.08 ± 0.65	2.03 ± 0.31	17.11 ± 2.13[ab]	48.75 ± 5.81[abc]
Post-implantation loss	4.20 ± 0.41	13.73 ± 1.57[a]	30.28 ± 4.19[ab]	46.69 ± 5.21[abc]
Fetal weight (g)	3.54 ± 0.41	3.46 ± 0.29	3.08 ± 0.37	2.53 ± 0.31
Placental weight (g)	0.67 ± 0.08	0.71 ± 0.09[a]	0.79 ± 0.19[a]	0.86 ± 0.12[a]
Crown-rump length (cm)	3.18 ± 0.19	3.01 ± 0.27	2.78 ± 0.31	2.61 ± 0.23

Value represents mean ± S.E. of 10 rats in each group.
The significance of the difference among various groups was evaluated by applying one-way ANOVA; Significance level: $p < 0.05$. Comparison between two groups: [a]vs. control; [b]vs. 250 ppm; [c]vs. 500 ppm.

2.2. Study of estrus cycle

Vaginal smears from 10 rats from each group were taken, once every morning, promptly spread on a clean slide and fixed in a solution of ethyl ether and ethanol. After staining with H and E, the slides were studied microscopically for quantification of epithelial cells and the frequency of cornified cells was calculated as a percentage. The intervals in days between two successive peaks in the frequency of cornified cells was taken as the length of each individual estrus cycle [18]. The study was continued for 12 consecutive estrus cycles.

2.3. Statistical analysis

Overall significance of differences in mean values between control and treatment groups was tested using one way ANOVA. Prior to the analysis, normality assumption of the data and homogeneity of variance between the experimental groups was ascertained. The means of the experimental groups from the controls and between two treatments were compared separately using Dunett's post hoc test [19]. Significance of difference in incidence of gross and skeletal abnormalities between group III and IV was tested using Fisher's Exact Test as the expected cell frequencies were less than five.

3. Results

No notable changes in behavior or clinical signs were observed in control or in treated dams. No mortality was observed during the experimental period. Daily water consumption in groups I, II, III and IV was 28.05, 25.78, 24.41 and 20.37 ml/rat/day, respectively. Based on this water intake, the chromium level reaching the treated groups (II, III and IV) was 6.44, 12.20 and 15.28 mg/rat/day. As the dose was increased, the mating index was found to be increasingly reduced. A similar pattern was seen with the fertility index which was calculated from the mated females (Table 1). Mothers of group IV and III registered a reduction in gestational weight gain (55.5 ± 3.01 and 60.92 ± 2.13 g, respectively). However, when compared with group I (controls), group IV and group III gained 21% and 14% less weight, respectively.

The number of corpora lutea was reduced in group III and group IV when compared to the control group (Table 1). The number of implantations was also significantly reduced in group III and IV when compared to controls. The number of fetuses per litter was significantly reduced in groups III and IV when compared to the control and the 250 ppm group (group II). However, when group III and IV were compared they did

Table 2
Incidences of gross and skeletal abnormalities in the pups of chromium-treated rats during the pregestational period

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Sub-dermal hemorrhagic patches	0	0	8/6 (16)	8/4 (42)[a]
Kinky tail	0	0	0	8/6 (42) [a]
Short tail	0	0	4/4 (9)	10/4 (53)[a]
Skeletal abnormalities				
Number of pups/litter observed	48/10	45/10	34/10	19/10
Reduced parietal ossification	0	0	0	12/10 (63)[a]
Reduced inter-parietal ossification	0	0	0	10/10 (53)[a]
Reduced caudal ossification	6/4 (12)	8/5(18)	18/8 (53)[a]	18/10 (95)[a]

Gross and skeletal abnormalities are represented as number of abnormal pups/litter observed; percentage in parentheses calculated by the total number of pups observed.
Statistical significance evaluated by Fisher's Exact test; comparison between two groups: [a]vs. control. Significance level: $p < 0.05$.

not show any marked difference. The number of resorption sites was found significantly increased in all the groups compared with controls. Pre- and post-implantation loss was also significantly increased in all the groups compared to controls (Table 1).

3.1. Gross abnormality

There were significant gross structural abnormalities in group IV in the form of sub dermal hemorrhagic patches on the thoracic and abdominal areas, as well as kinky and short tails (Table 2).

3.2. Visceral abnormality

No gross visceral abnormality was seen in any of the treated groups.

3.3. Skeletal abnormality

Significant increases in the incidence of reduced ossification in parietal, interparietal and caudal bones were observed in the 750 ppm dosed group, whereas the 500 ppm dosed group revealed significant incidence of reduced ossification in caudal bones only (Table 2).

3.4. Chromium levels

Chromium levels were found to be significantly increased in the treated rats of group III and IV as evidenced by significantly higher metallic levels in maternal blood, placenta and fetuses (Table 3). The rate of transfer of chromium from the mother to placenta and from placenta to fetus, calculated as a ratio, revealed that in group IV the placental metal level was more than in the other treated groups which showed almost the same chromium transfer. The transfer ratio from placenta to fetus did not show any change in any of the treated groups.

3.5. Estrus cycle

The length of estrus cycle was increased due to chromium treatment in all the groups but was only significant in the highest dosed group (Table 4).

4. Discussion

In the present study, rats exposed to chromium through drinking water during the pregestational period revealed a reduced number of corpora lutea and implantations, retarded fetal develop-

Table 3
Chromium concentrations in different tissues of rats treated during the pregestational period

Tissue	Group I (control)	Group II (250 ppm)	Group III (500 ppm)	Group IV (750 ppm)
Blood (μg/ml)	0.034 ± 0.007	0.049 ± 0.006^{a}	0.059 ± 0.008^{a}	0.192 ± 0.007^{abc}
Placenta (μg/g: fw)	0.093 ± 0.001	0.151 ± 0.008^{a}	0.168 ± 0.002^{ab}	0.232 ± 0.019^{abc}
Fetus (μg/g: fw)	0.042 ± 0.008	0.069 ± 0.007	0.163 ± 0.013^{ab}	0.241 ± 0.011^{abc}

Values represent mean ± S.E. of five rats in each group; fw, fresh weight.
Significance of the difference among various groups was evaluated by applying one-way ANOVA; Significance level: $p<0.05$; comparison between two groups: [a]vs. control; [b]vs. 250 ppm; [c]vs. 500 ppm.

ment and embryo- and fetotoxic effects as evidenced by the reduced number of fetuses (live and dead) per dam and higher incidence of still births, pre- and post-implantation loss in 500 and 750 ppm dosed mothers.

In our earlier study [8], a complete absence of implantation in 750 ppm treated mice was noted though reduced ovulation was present as evidenced by the significantly reduced number of corpora lutea. Mating was noticed in the 750 ppm group indicating that chromium treatment did not drive all the mice acyclic. The present study shows a species difference in the sensitivity between rats and mice. The chromium exposure (750 ppm) to mice showed a more significant effect on the duration of estrous cycle (72%) as compared to that in rats (37%). A differential effect on the implantation has also been observed in the two species but no correlation has been established yet.

The litter size in the 500 and 750 ppm dose groups was significantly reduced. This may be due to the effect of chromium (VI) on preimplantation embryos as evidenced by the study of Jacquet and Draye [20]. The maternal chromium is reported to pass freely through the placenta to the growing fetus as evidenced earlier from the analysis of bones from 120 human embryos in which the chromium content increased with age [7]. The levels of chromium used in the present study are not usually found in the environment but may be encountered at the work place or in effluents from the industrial establishments (40–50 000 ppm) [1].

Earlier studies have reported impaired gestational development when chromium was administered parenterally. Gale [21] injected 8 mg chromium trioxide per kg intravenously in hamsters on day 8 of gestation and found increased incidence of cleft palate.

In the present study, chromium accumulation in the fetuses of the 500 and 750 ppm groups might be attributed to the excessive transfer from maternal blood through placenta to fetus as evidenced by the placental/fetal chromium ratio. Therefore, the impaired fetal physiology in group III and IV resulting in embryo- and fetotoxic effects might be due to chromium accumulation as also seen with other heavy metals (Hg, Cd) and other xenobiotics [22]. Chromium (VI) is more readily transferred to the embryo and fetus [6,23] and is reported to produce teratogenic effects probably due to higher embryonic concentration [23].

The length of the estrus cycle was significantly increased in the highest dosed group (750 ppm). This might be correlated with the reduced number of ovulations observed in the highest dosed group as has already been reported and explained for chromium (VI) [24] and other chemicals [18]. The length of estrus cycle was also prolonged due to cadmium administration as reported by Baranski and Sitarek [25].

Danielsson et al. [23] studied the embryonic and fetal levels of chromium in early and late gestational stages of mouse and reported high placental chromium and increased passage to the fetus thereby affecting directly the embryonic structures. The lack of any marked teratological changes in the pre ent study compared to other investigators who exposed dams through parenteral administration, may be due to diminished uptake of chromium through the intestinal wall [26], when administered through drinking water. The absorption of some chromium through the

Table 4
Effect of chromium on the duration of estrus cycle

	Group I (control)	Group II (250 ppm)	Group III (500 ppm)	Group IV (750 ppm)
Estrus cycle (days)	5.2 ± 0.2	5.4 ± 0.7	5.7 ± 0.6	7.1 ± 0.5^a

Value represents mean ± S.E. of 10 rats in each group.
Significance of the difference among various groups was evaluated by applying one-way ANOVA; significance level: $*p<0.05$; comparison between two groups: [a]vs. control.

intestine in experimental animals and humans is well documented [27] and chromium (VI) is absorbed to a greater extent than chromium (III) through the gastro-intestinal tract [28]. Coogan et al. [29] reported higher tissue levels of chromium (VI) compared to chromium (III) which reflects the greater tendency of chromium (VI) to traverse the plasma membrane and bind to the intracellular protein in various tissues, and this may explain the greater degree of toxicity associated with chromium (VI). Embryonic and fetal levels of chromium (VI) after chromate exposure to pregnant rats is reported to be 10 times greater [23] than that found after exposure to corresponding doses of chromium (III).

Therefore, the present study indicates that sufficiently high chromium (VI) intake through drinking water pregestationally affects the embryonic and fetal development in rats and mice differently, the latter being more sensitive. Pregestational exposure causes deleterious effects during the process of preimplantation embryonic development and thereby implantation, while exposure during later development increases the number of resorbed and dead fetuses.

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Embryo- and Fetotoxicity of Chromium in Pregestationally Exposed Mice

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Chromium, an essential element in the human body required for proper carbohydrate, protein, and fat metabolism, is reported to impair gestational development of offspring of workers chronically exposed to this metal in the work place. Workers in chromium based industries can be exposed to concentrations two orders of magnitude higher than the general population (Hemminki and Vainio 1984). Among the general population, residents living near chromate production sites may be exposed to high levels of chromium (VI) in air or to elevated levels (40 - 50,000 ppm) of chromium in effluents (Rumar 1987). Shmitova (1978,1980) reported afterbirth and puerperal hemorrhages in women industrially exposed to this metal and observed high chromium levels in blood and urine of pregnant women and in fetal and cord blood. Chromium readily passes the placental barrier and reaches the growing fetus (Tipton 1960; Pribluda 1963). Exposure of mice to chromium during various gestational periods resulted in embryo and fetotoxic effects (Junaid et al. 1995, 1996).

Pribluda (1963) reported that the chromium content of bones of pregnant rats decrease with the advancement of gestation. Such released chromium may reach the circulatory system and enter feto-placental tissue through the placental barrier. Therefore, it was thought worthwhile to ascertain the role of body chromium accumulated pregestationally on embryo and fetal development and its subsequent transfer to feto-placental sites.

MATERIALS AND METHODS

Sixty, 4-month old, Swiss albino, female mice (body weight 30 ± 5 gms) of proven fertility from the Industrial Toxicology Research Centre colony were divided into four groups of fifteen mice each. Group I was given drinking water and served as the control, while groups II, III, and IV were treated with 250, 500 and 750 ppm chromium (VI, as potassium dichromate), respectively, in drinking water for 20 days [time required for complete development of an ovarian follicle (Pederson 1970]. The selection of doses was based on our earlier study (Trivedi et al. 1989) and the fact that the average chromium intake of humans is approximately 200 ug/day in drinking water (NRC 1989). The animals were individually housed

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under standard animal house conditions (room temprature 20-22°C, relative humidity 50±5%) where a regular cycle of 12 hrs light: 12 hrs darkness was maintained and were provided with feed pellets (Lipton India Ltd.) and water ad libitum. The dams were observed daily for water intake and clinical signs of toxicity. After 20 days, females were mated with normal healthy, adult males and females were checked for pregnancy the next morning. The day that the vaginal plug was found was designated as '0'day of gestation. Mothers were weighed and kept individually in plastic cages. Ten pregnant females were randomly selected from each group, weighed, and sacrificed on the 19th day of gestation under ether anesthesia and caesarian sections performed. Blood from five animals from each group was withdrawn from the heart in heparinized vials and kept at -20°C for chromium estimation. One fetus plus placenta/litter was also kept at -20°C for chromium estimation. Both ovaries were removed and the number of corpora lutea was determined. Total implantations, the number of fetuses/litter, the number of live/dead fetuses, crown-rump length, the number of resorptions, and the weight of the fetuses and their respective placenta were recorded. Pre and post-implantation loss (%) was calculated as described by Palmer et al. (1978). Remaining fetuses were examined for gross external abnormalities and 1/3 of these fetuses were fixed in Bouin's fluid for examination of visceral abnormalities (Wilson 1965), while the others were fixed in 95% ethanol, eviscerated, and stained by the Alizarin red S method (Staples and Schnell 1964) for examination of skeletal deformities (Kelsey 1974).

Known amounts of maternal blood, placentae and the fetuses were digested in Nitric acid:Perchloric acid (6:l) mixture till a white residue remained at the bottom of the flask. The residue was dissolved in 5.0 ml of 0.1 N Nitric acid and read on DC Plasma Emission Spectrophotometer (Beckman Spectrospan V). Blank and spiked samples were also run and analyzed simultaneously (Trivedi et al. 1989). The embryo- and feto-toxicity data in Table 1 and chromium estimation data in Table 3 were analysed by one-way ANOVA followed by Student's 't' test while gross and skeletal abnormalities data in Table 2 were analysed by Fischer's Exact Test (Brunning and Kintz 1977).

RESULTS AND DISCUSSION

The treated females did not show any notable change in behaviour or external features. Mortality (3 females; 20%) was observed in group IV. Autopsy of these animals could not establish the cause of death. Daily chromium (VI) intake as calculated by water consumed: 1.9 ± 0.02, 3.56 ± 0.03, and 5.23 ± 0.07 mg Cr for groups II, III, and IV, respectively. Water consumption in the control group was 8.52 ± 0.21 ml/mouse/day. No significant change in the weight of the mothers during the treatment was observed. Gestational weight gain of mothers in groups II and III was not significantly different when compared to controls; group IV registered no weight gain during gestation.

We observed an absence of implantation in the uterine horns of group IV mothers. While corpora lutea were present, their numbers were significantly reduced compared to the rest of the treatment groups.

Group III had a significant ($P<0.05$) increase in the number of resorptions (37%) when compared with the control group. Decrease in fetal weight (39%) and crown rump length (28%) and increase in placental weight (63%) as well as pre-(25%) and post-implantation (37%) loss was evident in group III compared to the control group. No significant difference in the number of corpora lutea was observed in group III compared to group II.

There was a significant ($P<0.05$) decrease in fetal weight (30%), placental weight (7%) and crown-rump length (17%) and an increase in post-implantation loss (18%) in group II compared to the control (Table 1). No dead fetuses were observed in any of the treated groups.

The fetuses of group III had higher ($P<0.05$) number of sub-dermal haemorrhagic patches and kinky and short tails. The number was markedly higher than for the control and group II animals (Table 2)

No major skeletal abnormalites was observed in any of the treated groups. Significantly reduced ossification in caudal, parietal and interparietal bones of the fetuses of group III was observed in treated mothers (Table 2). Soft tissue examination did not reveal any significant deformities in any of the treated groups.

Blood chromium was signifcantly higher in group IV compared to all other groups whereas that of groups II and III was elevated compared to controls. Placental chromium concentration increased in a dose-dependent manner in groups II and III compared to controls. Fetuses of mothers in group III had significantly higher chromium concentrations compared to fetuses of control and group II mothers (Table 3).

Chromium (VI) is reported to pass the placental barrier and accumulate in fetal tissues (Shmitova 1980). The presence of chromium (VI) in fetuses and infants has been reported in women working or living near the dichromate industries (Shmitova 1978). It was also noticed that women working in chromium-based industries for many years experienced abnormal menses, which was attributed to ovarian-hormonal impairment (Ross 1978). Tipton (1960) reported the transfer of chromium from the mother to the bones of the developing fetus in humans. In rats, the pregestationally retained chromium is reported to pass to the developing fetuses if exposure is stopped during gestation (Pribluda 1963).

Chromium speciation, concentration, and duration of exposure are important variables influencing tissue distribution. Gastrointestinal uptake of chromium is 2 - 10 % of the dose in both humans and laboratory animals. Shiraishi and Ichikawa (1972) reported that the bones and kidneys of rats contained the highest chromium concentration in comparison to other tissues monitored following oral administration of chromium (VI).

Table 1. **Chromium-induced embryo- and feto-toxicity in mice treated during the pregestational period.**

Parameters	Group I (Control)	Group II (250 ppm)	Group III (500 ppm)	Group IV (750 ppm)
Weight gain in mothers (g)	14.40 ± 1.01	13.43 ± 0.50	12.38 ± 0.49	1.7 ± 0.93
Number of corpora lutea/mice	7.9 ± 1.01	7.4 ± 0.50	7.3 ± 0.37	4.4 ± 0.50 abc*
Number of implantations/mice	7.7 ± 0.74	6.8 ± 0.41	5.4 ± 0.27 a*	0
Number of live fetuses/mice	7.7 ± 0.74	5.6 ± 0.50	3.4 ± 0.24 ab*	0
Number of resorptions/mice	0	1.20 ± 0.44	2.0 ± 0.31 a*	0
Pre-implantation loss (%)	2.77 ± 1.21	8.38 ± 3.53	24.79 ± 2.17 ab*	100 %
Post-implantation loss (%)	0	17.51 ± 2.22 a*	36.66 ± 4.94 ab*	0
Fetal weight (g)	1.59 ± 0.04	1.11 ± 0.04 a*	0.97 ± 0.03 ab*	0
Placental weight (g)	0.137 ± 0.003	0.128 ± 0.005a*	0.223 ± 0.005ab*	0
Crown-rump length (cm)	2.92 ± 0.07	2.41 ± 0.08 a*	2.09 ± 0.08 ab*	0

Value represents mean ± S.E. of 10 female mice in each group.

The significance of the difference among various groups was evaluated by applying one-way ANOVA followed by Student's 't' test (Brunning and Kintz 1977).

* Significance $p < 0.05$. Comparison between two groups: a -vs control; b -vs 250 ppm; c -vs 500 ppm

Table 2. Incidences of gross and skeletal abnormalities in the pups of dams treated with chromium during the pregestational period.

Parameters	Group I (Control)	Group II (250 ppm)	Group III (500 ppm)
Gross abnormalities			
Number of pups/litters observed	72/10	51/10	19/10
Drooping rist	0/10	0/10	6/4 (32)
Sub-dermal hemorrhagic patches	0	8/6 (16)	8/4 (42) a*
Kinky tail	0	0	8/6 (42) a*
Short tail	0	4/4 (9)	10/4 (53) a*
Skeletal abnormalities			
Number of pups/litter observed	48/10	34/10	19/10
Reduced parietal ossification	0	0	12/10 (63) a*
Reduced inter-parietal ossification	0	0	10/10 (53) a*
Reduced caudal ossification	6/4 (12)	18/8 (53) a*	18/10 (95) a*

Gross and skeletal abnormalites are represented as number of abnormal pups/litters observed.

The statistical significance was evaluated by Fisher's Exact test (Drunning and Kintz 1977).

Percentage in parentheses calculated by the total number of pups observed.

* Significance $p < 0.05$. Comparison between two groups: a-vs control.

Table 3. Chromium concentrations in different tissues of mice treated during the pregestational period

Tissue	**GroupI** (Control)	Group II (250 ppm)	Group III (500 ppm)	Group IV (750 ppm)
Blood (µg/mL)	0.03 ± 0.007	0.05 ± 0.006 a*	0.06 ± 0.008 a*	0.13 ± 0.007 abc*
Placenta (µg/g:f.w.)	0.09 ± 0.001	0.14 ± 0.008 a*	0.17 ± 0.002 ab*	No implantation
Fetus (µg/g:f.w.)	0.04 ± 0.008	0.07 ± 0.007	0.16 ± 0.013 ab*	No implantation

Values represent mean ± S.E of 5 mice in each group.

The significance of the difference among various groups was evaluated by applying one-way ANOVA followed by Student's 't' test (Brunning and Kintz 1977). * Significance $p < 0.05$.

Comparison between two groups: a -vs control; b -vs 250 ppm; c -vs 500 ppm. f.w. q fresh weight.

In the present study, the treated animals showed an increase in blood chromium concentration compared to controls, with the highest dose group (750 ppm) having the highest chromium concentrations. However, blood chromium concentrations of the 250 and 500 ppm dose group were not significantly different from one another. This may be attributed to the fact that chromium (VI) enters the red blood cells where reduction to chromium (III) and subsequent binding to hemoglobin takes place. Assimilation of chromium (VI) in excess of the amount that can be reduced and sequestered results in longer residence time of chromium (VI) in blood and, hence, greater exposure of body tissues (Saner 1980). Although we have not assessed the extent of chromium transfer from maternal tissues to fetal tissue in the present study, the results from previous studies suggest transfer of prestored chromium from maternal soft tissue and/or bones to the developing fetus (Fitzgerald et al. 1985).

We observed a dose-dependent rise in placental chromium concentration as compared to the fetus. This may be due to the placenta acting as a barrier to retard passage of chromium from the mother to the fetus to safeguard fetal development and growth. The highest close group in this study (750 ppm) did not show any implantation. However, the release of ovum. as evidenced by the presence of corpora lutea, was apparent, although highly reduced in number compared to the rest of the treated and control groups. This reduction in number of' corpora lutea may possibly be due to direct accumulation of chromium in ovarian tissue (Langard 1982) or reduced hormone levels (Mattison et al. 1983).

Pre-implantation loss (100%) in the highest dose group may also be attributed to reduced hormone levels (Mattison et al. 1983) or impaired embryos, as reported earlier (Jacquet and Draye 1982). Chromium passed to the fetus could have resulted in reduced fetal ossification, influencing fetal development either through a direct effect on fetal tissue (Matsumoto et al. 1976) or impairment of placental physiology (Faulk 1981).

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Bohn, Brent

From: Khan, Elaine@OEHHA <Elaine.Khan@oehha.ca.gov>
Sent: Wednesday, January 14, 2015 5:06 PM
To: Sasso, Alan
Cc: Gibbons, Catherine
Subject: RE: hypochlorhydria (high stomach pH) in the US population

Hi, Alan.
Thanks for the info. It looks very interesting. Unfortunately, I've never heard of the condition and I'm racking my brain trying to think if I would know a good person to ask about this. Please let me know if you find out more and vice versa.
Thanks!
Elaine

From: Sasso, Alan [mailto:Sasso.Alan@epa.gov]
Sent: Wednesday, January 14, 2015 1:54 PM
To: Khan, Elaine@OEHHA
Cc: Gibbons, Catherine
Subject: hypochlorhydria (high stomach pH) in the US population

Hi Elaine,

I really enjoyed the talk last week, thanks for sending us the info.

I was reading-up on gastric parameters in the human population (particularly as a function of fed/fasted status), and I saw in this Kalantzi paper, 2 out of the 19 subjects just happened to have a condition called "hypochlorhydria". They persistently have a very high stomach pH, and are very susceptible to gastric cancers and lesions/ulcers (due to biological/bacterial issues, infections, etc).

In 28 hypochlorhydric subjects (Feldman paper), the average basal pH was 7.44 in men,7.65 in women.

In 252 men WITHOUT hypochlorhydria (healthy, not taking medication, etc), 5% of them naturally had a basal/resting (fasted) gastric pH of at least 5.09. in women (n= 113), 5% had pH>=6.81. Those are conditions where our models indicate poor reduction.

So, even without hypochlorhydria, 10% of the population may be above pH=5 .

At the end of the Feldman paper, they say that the true incidence of hypochlorhydria in randomly selected adult humans in the US population is unknown (but that paper is from 1991). I'm having trouble obtaining information on what the incidence may be.

Have you ever heard of this condition?

-Alan

Alan F. Sasso, Ph.D.
Office of Research and Development
National Center for Environmental Assessment
U.S. Environmental Protection Agency

(703)-347-0179

Bohn, Brent

From:	Sasso, Alan
Sent:	Thursday, January 15, 2015 8:36 AM
To:	Khan, Elaine@OEHHA
Cc:	Gibbons, Catherine
Subject:	RE: hypochlorhydria (high stomach pH) in the US population

That's OK, it was more of an FYI than a question.

At 3 or 4 of our public meetings, we asked the public and industry to identify susceptible populations, and somehow nobody mentioned this!

-Alan

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Bohn, Brent

From: Khan, Elaine@OEHHA <Elaine.Khan@oehha.ca.gov>
Sent: Tuesday, July 02, 2013 1:35 PM
To: Gibbons, Catherine
Cc: Sasso, Alan
Subject: RE: NCEA/OEHHA Technical Meeting

Ok. Fyi, I've blocked off 2 hours just in case. We don't have to go that long (one hour might be plenty of time), but I thought I'd play it safe in case we get on a roll with things.

From: Gibbons, Catherine [mailto:Gibbons.Catherine@epa.gov]
Sent: Tuesday, July 02, 2013 9:32 AM
To: Khan, Elaine@OEHHA
Cc: Sasso, Alan
Subject: RE: NCEA/OEHHA Technical Meeting

That's great, thanks! I'll be in touch with a call-in number, I have to double-check the usage schedule. Thank you!

From: Khan, Elaine@OEHHA [mailto:Elaine.Khan@oehha.ca.gov]
Sent: Tuesday, July 02, 2013 11:53 AM
To: Gibbons, Catherine
Cc: Sasso, Alan
Subject: RE: NCEA/OEHHA Technical Meeting

10 am our time is perfect. We're looking forward to this! Thank you!

From: Gibbons, Catherine [mailto:Gibbons.Catherine@epa.gov]
Sent: Tuesday, July 02, 2013 8:52 AM
To: Khan, Elaine@OEHHA
Cc: Sasso, Alan
Subject: RE: NCEA/OEHHA Technical Meeting

Great! How about 10 am your time (and 1 pm here)? You can move this later, we are open for the rest of the day, shockingly!

From: Khan, Elaine@OEHHA [mailto:Elaine.Khan@oehha.ca.gov]
Sent: Tuesday, July 02, 2013 11:50 AM
To: Gibbons, Catherine
Cc: Sasso, Alan
Subject: RE: NCEA/OEHHA Technical Meeting

Hi, Catherine.

My schedule is wide open on July 8th, so if you want to propose a time for that day, I'd love to get our discussions started!

Elaine

From: Gibbons, Catherine [mailto:Gibbons.Catherine@epa.gov]
Sent: Tuesday, July 02, 2013 8:43 AM
To: Khan, Elaine@OEHHA
Cc: Sasso, Alan
Subject: RE: NCEA/OEHHA Technical Meeting

Hi Elaine!

Yes, we are very much looking forward to these discussions! Alan and I thought that setting up a meeting as soon as possible to at least provide an overview of issues and topics would be a good idea, even if we don't have a lot of time to devote to a specific review yet. I know you have a meeting on July 10; would it be better to meet before or after this date? The week of July 8 looks tight, but there is a lot of availability on our end on Monday July 8.

Thanks so much!

Catherine

Catherine Gibbons, Ph.D.
Biologist, IRIS Program
National Center for Environmental Assessment
USEPA Office of Research and Development
1200 Pennsylvania Ave. NW (8601P), Washington, DC 20460
Fed Ex/Physical Location: Two Potomac Yard (North Building), 2733 S. Crystal Drive Ste. N-7215, Arlington, VA 22202
Office (703) 603-0704 - Fax (703) 347-8689 - Cell (951) 288-2396

From: Khan, Elaine@OEHHA [mailto:Elaine.Khan@oehha.ca.gov]
Sent: Monday, July 01, 2013 5:19 PM
To: Gibbons, Catherine
Subject: NCEA/OEHHA Technical Meeting

Hi, Catherine.

It was great meeting with you and discussing some of our risk assessment technical issues. I look forward to our future meetings to discuss some issues in more detail. Please let me know your availability when you have a better idea of what your schedule will look like in the coming weeks. Thanks!

Elaine

Elaine M. Khan, Ph.D., Chief
Water Toxicology Section
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California Environmental Protection Agency
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Bohn, Brent

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